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Differences in the Association Between Persistent Pathogens and Mood Disorders Among Young- to Middle-Aged Women and Men in the U.S.

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Abstract

Background. A growing literature supports the role of immune system alterations in the etiology of mood regulation, yet there is little population-based evidence regarding the association between persistent pathogens, inflammation and mood disorders among younger women and men in the U.S. **Methods.** We used data from the National Health and Nutrition Examination Survey III on individuals 15-39 years of age assessed for major depression, dysthymia, and/or bipolar disorder I and tested for cytomegalovirus (N=6825), herpes simplex virus (HSV)-1 (N=5618) and/or *Helicobacter pylori* (*H. pylori*) (N=3167) seropositivity as well as C-reactive protein (CRP) level (N=6788). CMV immunoglobulin G (IgG) antibody level was also available for a subset of women (N=3358). We utilized logistic regression to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between pathogens, CRP levels and each mood disorder overall and among women and men, separately. **Results.** *H. pylori* seropositivity was associated with increased odds of dysthymia (OR 2.37, 95% confidence interval (CI): 1.07, 5.24) among women, but decreased odds among men (OR 0.51, 95% CI: 0.28, 0.92). CMV seropositivity was also associated with lower odds of depression (OR 0.54, 95% CI: 0.32, 0.91) among men, while elevated CMV IgG level was marginally associated with increased odds of mood disorders among women. Associations were not mediated by CRP level. **Conclusions.** Our findings suggest that persistent pathogens such as CMV and *H. pylori* may differentially influence mood disorders among women and men, warranting further investigation into biological and/or sociocultural explanations for the contrasting associations observed.

1. Introduction

More than 20% of U.S. adults between the ages of 18-49 have experienced a mood disorder such as major depressive disorder, dysthymia, or bipolar disorder in their lifetime (Kessler et al., 2005). Individuals who experience mood disorders are more likely to engage in substance abuse (Regier et al., 1990), have other co-morbid medical conditions (Forty et al., 2014; Kang et al., 2015) and experience poorer psychosocial functioning across the life course (Coryell et al., 1993; Mehta et al., 2014; Rhebergen et al., 2010), with depression predicted to be the leading cause of disability worldwide by 2030 (Mathers, 2008). While much research has focused on identifying genetic and behavioral risk factors for the onset of mood disorders (Hirschfeld, 2002), the role that interactions between the immune system and the brain may play in mood regulation, has been increasingly recognized (Dantzer et al., 2008; Dantzer et al., 2011; Hamdani et al., 2013; Muneer, 2016). It is now well-understood, for example, that inflammation plays a key role in inducing symptoms commonly referred to as “sickness behavior” that overlap with those of depression (Dantzer et al., 2008; McCusker and Kelley, 2013) and in regulation of glutamatergic neurotransmission important for regulation of mood in both depression and bipolar disorder (Dantzer et al., 2011; Machado-Vieira et al., 2012) (Barbosa et al., 2014). Specifically, microglia in the brain are activated by inflammatory cytokines that cross the blood-brain barrier, altering astroglial function such that these cells produce increased levels of cytokines as well as glutamate, contributing to enhanced tryptophan metabolism via the indoleamine 2,3-dioxygenase pathway (Barbosa et al., 2014; Dantzer et al., 2011; Machado-Vieira et al., 2012). Imbalances in the metabolites of kynurenine which serve as either N-methyl-D-aspartate agonists or

antagonists and decreased serotonin production, in turn, may contribute to the onset of manic and depressive symptoms (Barbosa et al., 2014; Dantzer et al., 2011; Machado-Vieira et al., 2012). Microglial activation in the brain may also contribute to neural damage implicated in the etiology of depression and bipolar disorder (Muneer, 2016; Valkanova et al., 2013).

While acute infections may induce short-term increases in inflammation, researchers have hypothesized that persistent pathogens such as herpesviruses may serve as particularly salient infections in the etiology of mood disorders (Miller et al., 2005; Phillips et al., 2008; Prossin et al., 2015; Rector et al., 2014; Simanek et al., 2014; Trzonkowski et al., 2004). Once acquired, such pathogens are never cleared from the body and consequently may repeatedly stimulate the immune system over time, during periods of reactivation (Glaser R, 1994). Existing studies examining the association between herpesviruses including cytomegalovirus (CMV) and herpes simplex virus (HSV)-1 and outcomes such as depression and bipolar disorder have however, been primarily been conducted among older populations from specific geographic regions (Miller et al., 2005; Phillips et al., 2008; Prossin et al., 2015; Rector et al., 2014; Simanek et al., 2014; Trzonkowski et al., 2004) and findings regarding the mediating role of inflammation in the pathway between such pathogens have been mixed (Miller et al., 2005; Rector et al., 2014; Simanek et al., 2014; Trzonkowski et al., 2004). *Helicobacter pylori* (*H. pylori*), a gram-negative bacteria that can lead to persistent infection of the gut is another pathogen thought to chronically activate the immune system (Di Leo et al., 2005; Robinson et al., 2007). This pathogen has been linked to several gastrointestinal disorders with inflammation-related etiology (Ruggiero, 2010)

and a growing body of evidence also supports the role of interactions at the “gut-brain” axis in regulation of mood (Budzyński and Kłopocka, 2014; Deans, 2017; Evrensel and Ceylan, 2015). To our knowledge there are no studies, however, examining the association between *H. pylori* and mood disorders such as depression or bipolar disorder. Overall, there is a need to clarify whether persistent pathogens such as herpesviruses and *H. pylori* are associated with mood disorders in the younger, U.S. adult population and further examine the potential role of inflammation in mediating these associations.

Gender differences in prevalence and/or severity of mood disorders such as depression and bipolar disorder have also been well-documented (Diflorio and Jones, 2010; Piccinelli and Wilkinson, 2000) and increased incidence of conditions with inflammation-related etiology among women has recently been posited to explain this pattern (Derry et al., 2015; Diflorio and Jones, 2010; Piccinelli and Wilkinson, 2000). Women are more likely to be seropositive for and to have poorer cell-mediated immune control of herpesviruses (Dowd and Aiello, 2009; Dowd et al., 2009; Schillinger et al., 2004; Staras et al., 2006), which some studies have linked to elevated systemic inflammation (Bennett et al., 2012; Nazmi et al., 2010; Turner et al., 2014). While seroprevalence of *H. pylori* has been reported to be higher in men than women (Everhart et al., 2000), recent studies have identified differences in the composition of the gut microbiome between men and women which could serve to moderate the pathogenicity of *H. Pylori* (Dominianni et al., 2015; Haro et al., 2016). For example, Haro et al., identified higher levels of *Veillonella* and *Methanobrevibacter genera* in the gut microbiome of men compared to women, whereas levels of *Bacteroides* and

Bilophila was higher in women (Haro et al., 2016). *Bilophila wadsworthia* has been shown in animal models to promote Th1-dominated immune response (Devkota et al., 2012) and researchers have hypothesized that differential abundance of Th-promoting or inhibiting microbes could influence the host's inflammatory response to *H. pylori* infection (Rolig et al., 2013). Taken together, this suggests that the role of persistent pathogens in the etiology of mood disorders may differ for women compared to men.

This study utilizes data from a US national representative sample of young- to middle-aged adults to 1) examine the association between CMV, HSV-1 and *H. pylori* seropositivity as well as CMV immunoglobulin G (IgG) antibody levels (available among women only) and lifetime history of mood disorder outcomes including major depression, dysthymia and bipolar disorder I, 2) examine the mediating role of inflammation in these associations, and 3) assess whether these associations differ between women and men.

2. Materials and Methods

2.1. Study Population

Data come from the National Health and Nutrition Examination Survey (NHANES) III (1988–1994); a population-based, multistage stratified probability survey which collects information on the health and nutrition of the United States, civilian, non-institutionalized population. The survey was carried out by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention, from 1988 to 1994 and is designed to be representative of the US population. All participants in NHANES III provided informed consent at the time of enrollment. Only individuals 15-39 years of age were assessed for lifetime history of one or more mood disorders including

major depression, dysthymia and bipolar disorder I and II via the Diagnostic Interview Schedule, a structured psychiatric interview that employs DSM-III criteria (N=8449). Our sample was further limited to those that were also tested for seropositivity for CMV (N=6825), HSV-1 (N=5618) and/or *H. pylori* (N=3167) seropositivity as well as C-reactive protein (CRP) level (N=6792) and missing no data on covariates of interest. In addition, CMV immunoglobulin G (IgG) optical density (OD) values were available for 3358 women tested for CMV seropositivity. Individuals categorized as "Other" race/ethnicity were excluded due to limited sample size for this subgroup.

2.2. Laboratory Analyses

CMV specific IgG was measured by a commercially available Enzyme Linked Immunosorbent Assay (ELISA) (Quest International, Inc., Miami, FL). Sera with values near the ELISA cutoff were confirmed with a second ELISA assay (bioMerieux, Inc., Durham, NC). If the results from the first two tests disagreed, an Immunofluorescence Assay (IFA) (Bion International, Inc., Park Ridge, IL), was used and results from this test were provided as the final seropositivity test result. The sensitivity and specificity of these tests have been estimated to be 98 and 99%, respectively (Staras et al., 2006). Optical density (OD) values from the first ELISA test were available for a subset of women tested for CMV seropositivity (N=3435). OD values ≥ 3.0 were top-coded and women tested for CMV were categorized as having high (i.e., ≥ 3.0) or low (i.e., < 3.0) OD values. HSV-1 seropositivity was assessed by solid-phase enzymatic immunodot assays using purified glycoprotein gG-1 of HSV-1 as the antigen.(Schillinger et al., 2004) These immunodot assays have also been shown to have high sensitivity and specificity (Lee et al., 1986). In individuals ages 6-19 years of age from phase 1 (1988–

1991) of the study, IgG antibodies to *H. pylori* were measured via an enzyme-linked immunoassay (ELISA) (Pylori Stat, Whittaker Bioproducts, Walkersville, MD) in 1993 and in individuals ≥ 20 years of age from phase 1 via a different ELISA in 1996 (Wampole Laboratories, Cranbury, NJ) (Statistics., 2006).

Sera collected for the purpose of CRP testing were stored at -70 C and analyzed within 2 months using a modification of the Behring Latex-Enhanced CRP assay on the Behring Nephelometer Analyzer system™ (Behring Diagnostics, Westwood, MA). Both within and between-assay quality control procedures were used and the coefficient of variation was 3.2%–16.1% through the period of data collection. The limit of detection for CRP was 0.3 mg/dL (Gunter, 1996) and individuals were categorized as having low (i.e., < 0.3 mg/DL) or high (≥ 0.3 mg/dL) levels.

2.3. Outcomes

During the physical examination, individuals 15-39 years of age were assessed as ever having met the criteria for several mood disorder diagnoses including major depression, dysthymia, bipolar disorder type I and atypical bipolar disorder (i.e., bipolar type II) via the Diagnostic Interview Schedule, a structured psychiatric interview based upon the Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (DSM-III) criteria (Association, 1980). Individuals were categorized as having a lifetime history or no history of each mood disorder outcome.

2.4. Covariates

Covariates hypothesized as potential confounders of the association between persistent pathogens and mood disorders included age at interview (years), race/ethnicity (Non-Hispanic white, Non-Hispanic black, or Mexican-American), gender

(male or female), poverty income ratio (PIR, ratio of family's income to the federal poverty threshold per family size) (0-125%, >125-249%, >250%), smoking status (never; < 100 cigarettes in their lifetime, former; ≥ 100 cigarettes in their lifetime but did not smoke currently, or current smoker; ≥ 100 cigarettes in their lifetime and smoke currently, alcohol use (never drinker;<12 drinks in their lifetime or last 12 months, moderate drinker; >=12 drinks in last 12 months and <4 drinks per day for females or<5 drinks per day for males, on days they drank, or heavy drinker; >=12 drinks in last 12 months and ≥4 drinks per day for females or ≥5 drinks per day for males, on days they drank), body mass index (kg/m^2), and prescription medication use in the past month including non-steroidal anti-inflammatory drugs (NSAIDS), infection-related medications (i.e., antimicrobials, antivirals, antifungals and/or other antibiotics); and/or mood disorder-related medications (i.e., anti-depressant, anti-anxiety and/or anti-psychotics) according to the National Drug Code Directory prepared by the Product Information Management Branch of the Food and Drug Administration (Statistics, 1998.). Subjects who self-reported use of Advil, Nuprin, Medipren or Ibuprofen in the past month were also categorized as taking NSAIDS in the past month.

2.5. Statistical Analyses

Statistical analyses were performed using SAS v. 9.4 (SAS Institute Inc., USA). All analyses used appropriate weights and adjustments for strata and clustering to account for the complex survey design in NHANES III. We first estimated the weighted sociodemographic and behavioral characteristics of individuals assessed for mood disorder outcomes. We estimated the student's t-test to examine difference in mean values for continuous variables and the Rao-Scott Chi-square test for difference in

proportions for categorical variables comparing women and men. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between seropositivity for each pathogen as well as high CMV IgG antibody level (i.e., >3.0 optical density (OD) units) among CMV seropositive women (n=2307), and each outcome, adjusting for age, gender, race/ethnicity, PIR, BMI, smoking status, alcohol use, and use of infection-related or mood disorder-related medications. Individuals with missing data on any covariates of interest were excluded from all analyses. Logistic regression was also used to estimate the OR (95% CI) for the association between seropositivity for each pathogen as well as high CMV IgG antibody level among CMV seropositive women and CRP level, adjusting for age, gender, race/ethnicity, PIR, BMI, smoking status, alcohol use, and use of infection-related medications and/or NSAIDS. Last, we estimated the OR (95% CI) for the association between CRP level and each mood disorder outcomes in fully adjusted models in which medications controlled for included mood disorder-related medications and/or NSAIDS. Analyses were conducted for the full sample and stratified by gender. We also ran models including interaction terms between gender and pathogens (mood disorder and CRP level as outcome) or gender and inflammatory markers (mood disorders as outcome). Due to limited number of bipolar disorder II cases, we did not examine the association between exposures of interest and this outcome.

3. Results

Population-weighted sociodemographic and behavioral characteristics of individuals assessed for one or more mood disorders in the overall sample as well as among women and men, separately, are shown in Table 1. The mean (± SE) age of

individuals included in our sample was 27.6 (± 0.17), 51.9% were female, the majority were categorized as Non-Hispanic White (78.8%), and 20.5% had a PIR of <125%. The majority of individuals never smoked (53.3%), 19.4% were heavy drinkers and the mean (± SE) BMI of those included in our sample was 25.5 (± 0.13). Approximately 30% of individuals used NSAIDS in the past month, whereas 8.7% used infection-related medications and 1.6% used mood disorder-related drugs. Over half of individuals were HSV-1 seropositive (58.0%), whereas 46.5% and 23.0% of individuals were seropositive for CMV and *H. pylori*, respectively and 20.1% had elevated levels of CRP (≥ 0.3 mg/dL). Over half of individuals were HSV-1 seropositive (53.4%), whereas 46.6% and 23.0% of individuals were seropositive for CMV and *H. pylori*, respectively. Approximately 8.0% of individuals in our sample met the criteria for lifetime history of major depression, 6.0% for history of dysthymia and < 1% for history of bipolar disorder I. A greater proportion of men reported Mexican-American race/ethnicity, had a PIR >250%, were current smokers and reported heavy alcohol use, compared to women. In contrast, a greater proportion of women reported use of mood disorder-related and infection-related medications as well NSAIDS, were seropositive for CMV as well as HSV-1, had elevated CRP levels, lifetime history of major depression, and dysthymia, compared to men. Sample characteristics were similar among subsets of individuals tested for each pathogen (data not shown).

Table 2 shows the association between pathogens as well as CRP level and depression. There was no association between any pathogen and depression among the overall sample, nor among women, but there was a protective effect of CMV and *H. pylori* seropositivity on depression among men (OR 0.55, 95% CI: 0.33, 0.91 and OR

0.34, 95% CI: 0.2, 0.54, respectively, p-value for interaction = 0.0405 and 0.0037, respectively). Elevated CRP level was not associated with depression among the overall sample, nor among women, but among men, those with CRP level of ≥ 0.3 mg/dL had 2.78 (95% CI: 1.41, 5.48) times higher odds of depression, compared to men with levels < 0.3 mg/dL in in fully adjusted models (p-value for interaction = 0.0001).

The association between pathogens as well as CRP level and dysthymia is shown in Table 3. While CMV, HSV-1 and *H. pylori* seropositivity were associated with dysthymia in the overall sample (OR 1.42, 95% CI 1.02, 1.97, OR 1.57, 95% CI: 1.16, 2.12, and OR 1.85, 95% CI: 1.12, 3.07, respectively), these associations were attenuated and no longer statistically significant after covariate adjustment (see Table 3). Elevated CMV IgG antibody level was positively associated with dysthymia among CMV seropositive women (OR 1.78, 95% CI: 1.18, 2.84), however, the effect was slightly attenuated and no longer statistically significant in the fully adjusted model (OR 1.50, 95% CI: 0.93, 2.41). In analyses stratified by gender, *H. pylori* seropositivity was associated with dysthymia in the fully adjusted model (OR 2.47, 95% CI: 1.06, 5.73) among women, whereas *H. pylori* seropositivity was associated with a decreased odds of dysthymia among men (OR 0.51, 95% CI: 0.28, 0.92, p-value for interaction=0.0075). There was no statistically significant association between elevated CRP level and dysthymia among the overall sample or among women, but among men, the odds of dysthymia for those with elevated CRP level was 2.87 (95% CI: 1.56, 5.28) time higher compared to those with low CRP levels (p-value for interaction =0.0023).

Table 4 shows the association between pathogens as well as CRP level and bipolar disorder I. There was no statistically significant association between CMV, HSV-

1 or *H. pylori* seropositivity and bipolar disorder I among the overall sample, however, there was an association between this pathogen and bipolar disorder I in a protective direction among men (OR 0.24, 95% CI: 0.06, 0.91) in the fully adjusted model (p-value for interaction = 0.1156). While elevated CMV IgG antibody levels were also associated with bipolar disorder I among CMV seropositive women in the unadjusted model (OR 4.92, 95% CI: 1.17, 20.83), this association was slightly attenuated and no longer statistically significant after covariate adjustment (OR 4.34, 95% CI: 0.92, 20.45, p-value = 0.0623). There was a positive relationship between elevated CRP level and bipolar disorder I among the overall sample as well as among women and men, separately, but these associations were also not statistically significant (see Table 4).

The associations between each pathogen and elevated CRP level are illustrated in Figure 1. In crude models, CMV and HSV-1 seropositivity were associated with elevated CRP level in the overall sample (OR 1.40, 95% CI: 1.13, 1.74 and OR 1.32, 95% CI: 1.11, 1.59, respectively) and for women (OR 1.29, 95% CI: 0.99, 1.69 and OR 1.34, 95% CI: 1.07, 1.68, respectively), but these associations were attenuated and no longer statistically significant after covariate adjustment (see Figure 1). We consequently did not further assess the mediating role of CRP level in the associations between persistent pathogens and mood disorders.

4. Discussion

To our knowledge, this is the first study to examine the association between several persistent pathogens and an array of mood disorder outcomes among a population-based sample of younger US adults. We found that *H. pylori* seropositivity was associated with 2.5 times *higher* odds of dysthymia among women. Among men, in

contrast, *H. pylori* seropositivity was associated with decreased odds of both depression and dysthymia. While men with high versus low CRP levels had higher odds of depression, there were no associations between any pathogens and CRP in our study. Our findings suggest there is a need for further investigation into the biological as well as sociocultural mechanisms driving differences in the association between persistent pathogens and mood disorders between women and men in the general U.S. adult population.

We hypothesized that *H. pylori* may be involved in the etiology of mood disorders via inflammatory pathways, and that the inflammatory effects of this pathogen may differ for women compared to men. Our findings suggest however, that *H. pylori* may act outside of inflammatory pathways to differentially effect risk for mood disorders in women and men. As this is the first study, to our knowledge, to examine gender differences in the association between *H. pylori* and mood disorders, replication of our analyses in additional study populations as well as further investigation into drivers of the heterogeneous effects of *H. pylori* on mood disorders between women and men observed in our study are needed. Sex differences in the relationship between *H. pylori* and the orexigenic hormone, ghrelin (Chuang et al., 2009; Stec-Michalska et al., 2009), which is produced by endocrine cells in the gastrointestinal tract and has been linked in some studies to mood disorders (Zarouna et al., 2015) may serve as one explanation for our findings. For example, Chuang et al. recently demonstrated in a small cohort of dyspeptic patients (N=341), that *H. pylori* infection was associated with higher ghrelin levels among women (not statistically significant), but decreased levels among men (p=0.02) (Chuang et al., 2009). Some (Gecici et al., 2005; Ozsoy et al., 2014), although

not all (Zarouna et al., 2015), studies have identified higher levels of ghrelin in depressed individuals compared to those not depressed.

The finding of no association between CMV and HSV-1 seropositivity and depression is consistent with results from previous studies conducted among cohorts of middle-aged adults (Amsterdam and Hernz, 1993; Rector et al., 2014; Simanek et al., 2014). For example, Simanek et al. found no association between CMV or HSV-1 seropositivity and depression in a community-based sample of middle-aged (mean age 54.0 ± 15.8 years) Detroit residents (Simanek et al., 2014). Similarly, Rector et al. found no association between CMV seropositivity and depression among a cross-sectional sample of middle-aged (mean age 44) individuals in a German occupational cohort (Rector et al., 2014). The direction of the association between CMV IgG antibody level and mood disorder outcomes among women who were CMV seropositive, observed in our study was consistent, however, with findings from several previous studies examining this association with depression as well as bipolar disorder (Jaremka et al., 2013; Miller et al., 2005; Phillips et al., 2008; Prossin et al., 2015; Rector et al., 2014; Simanek et al., 2014; Trzonkowski et al., 2004), but only marginally statistically significant. CMV has been hypothesized as a major driver of aging of the adaptive immune system (Derhovanessian et al., 2009). Indeed, a recent study demonstrated that higher CMV IgG antibody levels were associated with an increase in the ratio of effector to naïve CD4 and CD8 T cell subsets among middle-aged adults living in Detroit (Aiello et al., 2016). A growing body of literature suggests that T cell function may, in turn, play an important role in the etiology of mood disorders (Barbosa et al., 2014; Duggal et al., 2014; Miller, 2010; Toben and Baune, 2015). It is possible that the

younger mean age of our study population may provide less variation in immune response to CMV compared to older populations, potentially dampening the association between CMV IgG antibody level and mood disorders among women in our study.

In contrast to our findings for women, we observed a statistically significant protective association between CMV seropositivity and mood disorders among men, suggesting that there may be gender differences in the effect of CMV on mood regulation between women and men. While CMV IgG antibody levels were only available for women in our sample and thus we could not assess whether there was variation in the effect of immune response to CMV on these outcomes between women and men, researchers have hypothesized that sex hormones (Dowd et al., 2013; van der Heiden et al., 2016; Zhu et al., 2000) and/or differential exposure to stress-mediated reactivation (Dowd and Aiello, 2009; Dowd et al., 2008) may play a key role in shaping differences in immune control of CMV between men and women. A recent study also suggests that the adverse effects of CMV on alterations to the T cell repertoire worsen for women over time (van der Heiden et al., 2016), warranting further investigation into whether variability in in the adverse effects of CMV infection on immune aging between women in men may partially explain the protective effect of CMV on mood disorders, observed among this population of younger men in our study is therefore warranted.

Similar to Ford et al. (Ford and Erlinger, 2004), we observed a strong positive association between elevated CRP and depression among men, but there was no statistically significant associations between any pathogens and CRP levels in the overall sample, nor among women and men separately. Thus, elevated CRP level did not meet the criteria as a mediator of the associations of interest. Our findings are

similar to those from the study by Simanek et al. in which the authors did not identify an association between CMV or HSV-1 seropositivity or HSV-1 IgG antibody level and levels of the pro-inflammatory markers CRP or interleukin-6, nor find evidence that these pro-inflammatory markers mediated the association between CMV IgG antibody level and depression onset (Simanek et al., 2014). Another recent study demonstrated that women who were exposed experimentally to an immune system challenge had a greater increase in depressed mood, compared to men, but that there were no differences in levels of TNF- α or IL-6 post-challenge, nor in the association between these pro-inflammatory markers and depressed mood between men and women (Moieni et al., 2015). It is possible that inflammation may serve as a more important mediator of the relationship between persistent pathogens such as CMV and mood disorders as individuals age via a process referred to as "inflammaging" (Baylis et al., 2013; Gruver et al., 2007). A recent study failed to find evidence for a causal association, however, between CMV and age-related changes in inflammation over time (Bartlett et al., 2012), warranting further clarification of the importance of inflammation as a mediator of the pathway between CMV as well as other persistent pathogens and mood disorder onset across the lifecourse.

A few limitations in the present study must be considered. First, NHANES III is a cross-sectional study, thus there is temporal ambiguity between the exposures and outcomes of interest. Indeed, while detection of IgG antibodies in the serum of individuals is a marker of previous exposure to the pathogens examined, we could not ascertain timing of acquisition of infection or frequency of reactivation of persistent pathogens in relation to mood disorder onset among individuals in our study. Moreover,

psychological distress resulting from adverse mental health conditions may also contribute to subsequent declines in immune function (Dhabhar, 2014). It is estimated that among those 12-19 years of age, the prevalence of CMV and HSV-1 is 41.7% and 44.4% (Schillinger et al., 2004; Staras et al., 2006), respectively and among those 15-19 years of age, seroprevalence of *H. pylori* is 29.1% (Staat et al., 1996). We carried out sensitivity analyses in which we excluded cases of depression and bipolar disorder I if symptom onset occurred before the age of 12 (N=43 and N=14, respectively) (data not available for dysthymia), finding that most effect estimates did not change by more than 10%, nor change in statistical significance (see Supplemental Tables 1 and 2). Future prospective studies in which incidence of mood disorders is ascertained from individuals who are free of mood disorders at baseline are needed, however, to better establish a causal association between persistent pathogens and onset of mood disorders over time.

Another limitation to our study is that mood disorder assessment was not based upon the most recent DSM criteria nor allow for redefining mood disorder classifications under the more recent National Institute of Mental Health Research Domain Criteria framework. Findings from this study lend support, however, for the potential inclusion of infection with persistent pathogens in the cluster of factors considered relevant under this framework. Numerous studies have demonstrated strong associations between socioeconomic factors and seropositivity as well as immune response to persistent pathogens (Dowd and Aiello, 2009; Dowd et al., 2009) and moreover, socioeconomic disparities in mood disorder outcomes such as depression (Lorant et al., 2003). In our study, we relied upon adjustment for socioeconomic position (SEP) at the household

level (i.e., PIR) as our sample combined young adults living within households as well as heads of households and educational attainment was unlikely to be completed for many of the individuals in our sample, and there could be residual confounding due to lack of control for individual SEP. It is unlikely, however, that residual confounding by socioeconomic factors would fully explain the associations observed as we also adjusted for several other factors that are correlated with individual level SEP including race/ethnicity, smoking status and BMI (Pampel et al., 2010). Data on IgG antibody levels was also limited to CMV and only available among women. In addition, CMV OD values among women tested for CMV seropositivity were top-coded and thus were dichotomized based upon an arbitrary cut-off point that may not correspond to an elevated immune response to CMV that is clinically relevant for these outcomes. Last, high sensitivity CRP testing was not performed in NHANES III and levels of CRP below the limit of detection were not available. Nonetheless, this study is, to our knowledge, the largest sample of individuals assessed for mood disorders and tested for persistent pathogens as well as inflammation and the only population-based study we are aware of that has examined these relationships with bipolar disorder.

5. Conclusions

To our knowledge, this is the first study to examine the association between several persistent pathogens and mood disorder outcomes among a population-based sample of young- to middle-aged women and men in the general U.S. population. Our findings, suggest that there may be important gender differences in the associations between CMV and *H. pylori* and mood disorders and that such pathogens may exert effects on these conditions outside of inflammatory pathways. Further examination of

social, biological and/or behavioral factors that may explain differences in the effect of persistent pathogens on mood disorders between women and men over time are therefore warranted.

Conflict of interest statement

All authors declare that there is no conflict of interest.

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Figure 1. Association Between Persistent Pathogens and C-reactive Protein Level Among U.S. Adults 15-39 Years of Age in the National Health and Nutrition Examination Survey III (1988-1994)

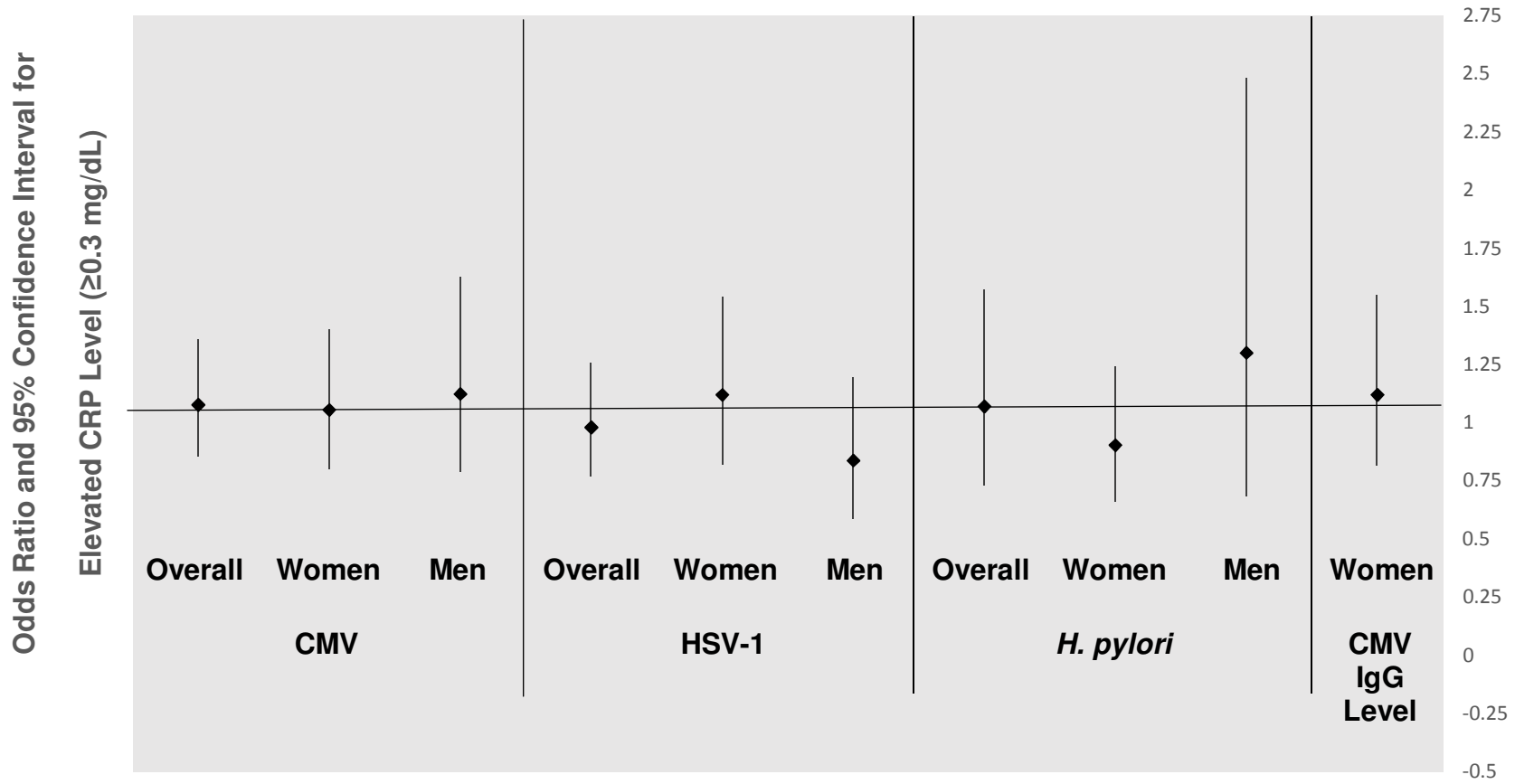

Table 1 Legend

CMV; cytomegalovirus, DSM-III; Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, HSV-1; herpes simplex virus-1, *H. pylori*; *Helicobacter pylori*, IgG; Immunoglobulin G, NSAID; non-steroidal anti-inflammatory drug.

[a]N=7230 for overall sample, N=3927 for women, N=3303 for men.

[b]N=7227 for overall sample, N=3918 for women, N=3309 for men.

[c]N=7251 for overall sample, N=3983 for women, N=3304 for men.

[d]N=6886 for overall sample, N=3747 among women, and N=3139 among men.

[e]IgG antibody levels among CMV seropositive women only.

[f]N=6828 for overall sample, N=2981 for women, and N=2683 for men.

[g]N=3185 for overall sample, N=1634 for women, and N=1551 for men.

[h]N=7345 for overall sample, N=3721 for women, and N=3107 for men.

[i]N=7294 for overall sample, N=3966 for women, and N=3325 for men.

[j]N=7292 for overall sample, N=3967 for women, and N=3325 for men.

*P<0.05.

Table 2 Legend

CMV; cytomegalovirus, IgG; immunoglobulin G, HSV-1; herpes simplex virus-1, *H. pylori*; *Helicobacter pylori*

[a]Models adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of mood disorder and/or infection-related medications. Models for C-reactive protein adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of mood disorder-related and/or non-steroidal anti-inflammatory medications.

[b]N=6813 for overall sample, N=3695 for women, and N=3118 for men.

[c]N=2306 for CMV seropositive women.

[d]N=5609 for overall sample, N=2945 for women, and N=2664 for men.

[e]N=3160 for overall sample, N= 1621 for women, and N=1539 for men.

[f]N=6776 for overall sample, N=3697 for women, and N=3097 for men.

*P<0.05

Table 3 Legend

CMV; cytomegalovirus, IgG; immunoglobulin G, HSV-1; herpes simplex virus-1, *H. pylori*; *Helicobacter pylori*

[a]Models adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of mood disorder and/or infection-related medications. Models for C-reactive protein adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of mood disorder-related and/or non-steroidal anti-inflammatory medications.

[b]N=6778 for overall sample, N=3696 for women, and N=3082 for men.

[c]N=2358 for CMV seropositive women.

[d]N=5618 for overall sample, N=2974 for women, and N=2671 for men.

[e]N=3167 for overall sample, 1621 for women, and N=1546 for men.

[f]N=6788 for overall sample, N=3702 for women, and N=3086 for men.

*P<0.05

**P<0.06

Table 4 Legend

CMV; cytomegalovirus, IgG; immunoglobulin G, HSV-1; herpes simplex virus-1, *H. pylori*; *Helicobacter pylori*

[a]Models for pathogens adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of mood disorder and/or infection-related medications. Models for C-reactive protein adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of mood disorder-related and/or non-steroidal anti-inflammatory medications.

[b]N=6823 for overall sample, N=3698 for women, and N=3125 for men.

[c]N=2358 for CMV seropositive women.

[d]N=5617 for overall sample, N=2946 for women, and N=2671 for men.

[e]N=3167 for overall sample, 1621 for women, and N=1546 for men.

[e]N=3167 for overall sample, 1621 for women, and N=1546 for men.

[f]N=6786 for overall sample, N=3700 for women, and N=3086 for men.

*$P<0.05$

Figure 1 Legend

CMV; cytomegalovirus, IgG; immunoglobulin G, HSV-1; herpes simplex virus-1, *H. pylori*; *Helicobacter pylori*

[a]Models adjusted for age, gender, race/ethnicity, poverty income ratio, smoking status, alcohol use, body mass index (kg/m^2) and use of infection-related and/or non-steroidal anti-inflammatory medications.

[b]N=6778 for overall sample, N=3696 for women, and N=3082 for men.

[d]N=5623 for overall sample, N=2960 for women, and N=2663 for men.

[e]N=3159 for overall sample, N=1624 for women, and N=1535 for men.

[c]N=2312 for CMV seropositive women.

*P<0.05

Table 1. Population weighted socioedemographic and behavioral characteristics of individuals 15-39 years of age assessed for mood disorders via DSM-III criteria in the National Health and Nutrition Examination Survey III (1988-1994)

Characteristics	Total Population (N=7294)	Women (N=3969)	Men (N=3325)	P-value
Age, years (mean ± SE)	27.6 ± 0.17	27.7 ± 0.24	27.6 ± 0.19	0.8836

Gender, N (%)				
Female	3969 (50.8)	---	---	----
Male	3325 (49.2)	---	---	
Race/Ethnicity, N (%)				
Non-Hispanic White	2277 (78.9)	1287 (78.5)	990 (79.3)	0.0007*
Non-Hispanic Black	2558 (13.9)	1426 (14.8)	1132 (12.9)	
Mexican-American	2459 (7.2)	1256 (6.7)	1203 (7.8)	
Poverty Income Ratio, N (%)				
0-125%	2744 (20.5)	1264 (22.4)	1177 (18.5)	0.0011*
>125-249%	2167 (27.6)	1138 (27.8)	1029 (27.5)	
> 250%	2383 (51.9)	1567 (49.8)	1119 (54.0)	
Smoking Status				
Never	4446 (53.3)	2625 (56.4)	1821 (50.1)	0.0012*
Former	812 (13.6)	379 (12.4)	433 (14.8)	
Current	2036 (33.1)	965 (31.2)	1071 (35.0)	
Alcohol Use, N (%)				

Never	3530 (38.1)	2434 (48.0)	1096 (27.8)	<0.0001*
Moderate	2443 (42.6)	1111 (39.6)	1332 (45.7)	
Heavy	1321 (19.4)	424 (12.4)	987 (26.5)	
Body mass index (kg/m^2) (mean ± SE)	25.2 ± 0.13	25.0 ± 0.18	25.5 ± 0.15	0.0458*
Mood Disorder-related Drug Use [a], N (%)				
No	7125 (98.4)	3857 (97.8)	3268 (99.1)	0.0075*
Yes	105 (1.6)	70 (2.2)	35 (0.9)	
Infection-related Drug Use [b], N (%)[b]				
No	6691 (91.3)	3547 (88.3)	3144 (94.4)	<0.0001*
Yes	536 (8.7)	371 (11.7)	165 (5.6)	

NSAID Use[c], N (%)				
No	5424 (69.1)	2710 (61.5)	2714 (77.0)	<0.0001*
Yes	1827 (30.9)	1273 (38.5)	590 (23.0)	
CMV Serostatus[d], N (%)				
Seronegative	2446 (53.5)	1144 (47.0)	1302 (60.0)	<0.0001*
Seropositive	4440 (46.5)	2603 (53.0)	1837 (40.0)	
CMV IgG Antibody Level[e]				
Low (≤ 3.0 OD units)		1282 (56.8)		----
High (>3.0 OD units)		1054 (43.2)		
HSV-1 Serostatus[f], N (%)				

Seronegative	1695 (42.0)	829 (38.8)	866 (45.3)	0.0299*
Seropositive	3969 (58.0)	2152 (61.2)	1817 (54.7)	
***H. pylori* serostatus[g], N (%)**				
Seronegative	1941 (77.0)	1041 (78.7)	900 (75.2)	0.1457
Seropositive	1244 (23.0)	593 (21.3)	651 (24.8)	
C-reactive Protein Level[h]				
Low (< 0.3 mg/dL)	5162 (79.9)	2564 (73.7)	2598 (86.4)	<0.0001*
High (≥ 0.3 mg/dL)	1666 (20.1)	1157 (26.3)	509 (13.6)	
Major Depression[i]				

No	6753 (92.0)	3585 (89.1)	3168 (94.9)	<0.0001*
Yes	528 (8.0)	379 (10.9)	149 (5.1)	
Dysthymia				
No	6764 (94.0)	3613 (92.3)	3151 (95.8)	<0.0001*
Yes	530 (6.0)	356 (7.7)	174 (4.2)	
Bipolar Disorder I[j]				
				0.8068
No	7222 (99.2)	3927 (99.2)	3295 (99.2)	
Yes	70 (0.8)	40 (0.8)	30 (0.8)	

Table 2. Association Between Persistent Pathogens, C-reactive protein and Lifetime History of Major Depression Among U.S. Adults 15-39 Years of Age in the National Health and Nutrition Examination Survey III (1988-1994)

	Odds Ratio (95% Confidence Interval)[a]			
	Total Population	**Women**	**Men**	**P-value**
Pathogens				
CMV Serostatus[b]				
Seronegative	1.0	1.0	1.0	
Seropositive	0.86 (0.64, 1.16)	1.09 (0.74, 1.58)	0.54 (0.32, 0.91)*	0.0405*
CMV IgG Antibody Level[c]				
Low (≤ 3.0 OD units)	--	1.0	--	
High (> 3.0 OD units)	--	1.25 (0.78, 2.04)	--	
HSV-1 Serostatus[d]				0.2520
Seronegative	1.0	1.0	1.0	
Seropositive	0.85 (0.61, 1.20)	0.97 (0.64, 1.48)	0.67 (0.41, 1.10)	
***H. pylori* Serostatus[e]**				0.0037*

Seronegative	1.0	1.0	1.0	
Seropositive	0.98 (0.46, 2.10)	1.76 (0.73, 4.28)	0.29 (0.13, 0.62)*	
C-reactive protein level[f]				
Low (< 0.3 mg/dL)	1.0	1.0	1.0	0.0001*
High (≥ 0.3 mg/dL)	1.17 (0.79, 1.75)	0.70 (0.46, 1.08)	2.97 (1.56, 5.64)*	

Table 3. Association Between Persistent Pathogens, C-reactive protein and Lifetime History of Dysthymia Among U.S. Adults 15-39 Years of Age in the National Health and Nutrition Examination Survey III (1988-1994)

	Odds Ratio (95% Confidence Interval)[a]			
	Total Population	**Women**	**Men**	**p-value**
Pathogens				
CMV Serostatus[b]				

Seronegative	1.0	1.0	1.0	0.5927
Seropositive	0.99 (0.70, 1.40)	1.21 (0.75, 1.69)	0.87 (0.46, 1.65)	
CMV IgG Antibody Level[c]				
Low	--	1.0	--	----
High	--	1.50 (0.93, 2.41)**	--	
HSV-1 Serostatus[d]				
Seronegative	1.0	1.0	1.0	0.3468
Seropositive	1.06 (0.74, 1.51)	0.90 (0.57, 1.46)	1.47 (0.76, 2.86)	
***H. pylori* Serostatus[e]**				
Seronegative	1.0	1.0	1.0	0.0075*
Seropositive	1.38 (0.65, 2.91)	2.47 (1.06, 5.73)*	0.51 (0.28, 0.92)*	
C-reactive protein level[f]				
Low (< 0.3 mg/dL)	1.0	1.0	1.0	0.0023*
High (≥ 0.3 mg/dL)	1.40 (0.89, 2.20)	0.92 (0.56, 1.50)	2.87 (1.56, 5.28)*	

Table 4. Association Between Persistent Pathogens and Lifetime History of Bipolar Disorder I Among U.S.

Adults 15-39 Years of Age in the National Health and Nutrition Examination Survey III (1988-1994)

	Odds Ratio (95% Confidence Interval)[a]			
	Total Population	**Women**	**Men**	**P-value**
Pathogens				
CMV Serostatus[b]				
Seronegative	1.0	1.0	1.0	0.1620
Seropositive	0.75 (0.31, 1.81)	1.43 (0.48, 4.26)	0.37 (0.07, 2.05)	
CMV IgG Antibody Level[c]				
Low	--	1.0	--	----
High	--	4.34 (0.92, 20.45)**	--	
HSV-1 Serostatus[d]				
Seronegative	1.0	1.0	1.0	0.5167
Seropositive	0.99 (0.47, 2.08)	1.31 (0.35, 4.86)	0.84 (0.31, 2.25)	
***H. pylori* Serostatus[e]**				
Seronegative	1.0	1.0	1.0	0.1556
Seropositive	0.64 (0.20, 2.10)	0.89 (0.35, 2.27)	0.24 (0.06, 0.91)*	

C-reactive protein level[f]				
Low (< 0.3 mg/dL)	1.0	1.0	1.0	0.3161
High (≥ 0.3 mg/dL)	2.13 (0.81, 5.64)	1.14 (0.34, 3.79)	3.29 (0.80, 13.53)	